# THE COMPOSITION AND PHYLOGENETIC SIGNIFICANCE OF THE *MOUGEOTIA* (CHAROPHYCEAE) CELL WALL[1]

*Arland T. Hotchkiss, Jr.*[2]

U.S. Department of Agriculture, Eastern Regional Research Center
600 E. Mermaid Lane, Philadelphia, Pennsylvania 19118

*Michael R. Gretz*

Department of Biology, George Mason University, Fairfax, Virginia 22030

*Kevin B. Hicks*

U.S. Department of Agriculture, Eastern Regional Research Center
600 E. Mermaid Lane, Philadelphia, Pennsylvania 19118

*and*

*R. Malcolm Brown, Jr.*

Department of Botany, The University of Texas at Austin, Austin, Texas 78713-7640

ABSTRACT

*The two-layered, fibrillar cell wall of* Mougeotia *C. Agardh sp. consisted of 63.6% non-cellulosic carbohydrates and 13.4% cellulose. The orientation of cellulose microfibrils in the native cell wall agrees with the multinet growth hypothesis, which has been employed to explain the shift in microfibril orientation from transverse (inner wall) toward axial (outer wall). Monosaccharide analysis of isolated cell walls revealed the presence of ten sugars with glucose, xylose and galactose most abundant. Methylation analysis of the acid-modified, 1 N NaOH insoluble residue fraction showed that it was composed almost exclusively of 4-linked glucose, confirming the presence of cellulose. The major hemicellulosic carbohydrate was semi-purified by DEAE Sephacel ($Cl^-$) anion-exchange chromatography of the hot 1 N NaOH soluble fraction. This hemicellulose was a xylan consisting of a 4-xylosyl backbone and 2,4-xylosyl branch points. The major hot water soluble neutral polysaccharide was identified as a 3-linked galactan.*

Mougeotia *cell wall composition is similar to that of* Klebsormidium *(Charophyceae) and has homologies with vascular plant cell walls. Our observations support ultrastructural evidence which suggests that members of the Charophyceae represent the phylogenetic line that gave rise to vascular plants. Therefore, the primary cell walls of vascular plants may have evolved directly from structures typical of the filamentous green algal cell walls found in the Charophyceae.*

*Key index words: cellulose; cell wall; Charophyceae;* Mougeotia; *phylogeny; xylan*

Our understanding of the plant cell wall is based mostly on information from vascular plants. The structural composition of the algal cell wall is relatively unknown except for the glycoprotein *Chlamydomonas* cell wall (Roberts et al. 1985) and for those algal cell walls from which economically important polysaccharides (agar, carrageenan, alginate and fucoidan) are derived (Percival and McDowell 1981). Therefore, it is uncertain whether vascular plant cell wall models (Albersheim 1975, Lamport and Epstein 1983, Cooper et al. 1984) are appropriate for algae.

Structural similarities have been observed between algal cell walls and those of vascular plants. Seigel and Seigel (1973) described basic algal cell wall architecture as consisting of a highly polymeric fibrillar framework interspersed with lower molecular weight polymers and oligomers (often as gel-like fibers) plus inorganic ions and supplemented

with hydrated colloidal arrays of polymers. This algal cell wall structure was thought to be consistent with the wall architecture observed in all nonpeptidoglycan plant cell walls, thus providing the basis for the proposal that the evolution of the eukaryotic cell wall was monophyletic (Seigel and Seigel 1973).

Although structural similarities exist, the composition of vascular plant and algal cell walls may be dissimilar (Preston 1968, Seigel and Seigel 1973). In some marine green and red algae, fibrillar 3-linked xylan or 4-linked mannan replace cellulose as the major load-bearing element of the cell wall (Preston 1974). Many marine algal cell wall colloids and mucilages are highly sulfated, whereas sulfation of vascular plant cell wall polysaccharides has not been reported (Percival and McDowell 1981). Except for cellulose, cell wall carbohydrates structurally identical to those typical of vascular plants have not been reported among the algae. Debate even exists concerning the crystallographic similarities between algal and vascular plant cellulose (Atalla and VanderHart 1984).

The great variation in morphology of algae is reflected in the structure of the cell wall or outer envelope (Bold and Wynne 1985). This structure can be absent, loricate, separated from the cytoplasm by mucilage, or composed of scales, intracellular pellicular strips or frustules. Cell walls in members of the Chlorophyceae *(Oocystis, Cladophora, Chaetomorpha)* and the Ulvophyceae *(Valonia, Boergesenia)* are lamellate consisting of layers of helical microfibrils displaying an interlayer shift in orientation up to 90° (Preston 1968, 1974). The helicoidal pattern of cell wall microfibril orientation (Neville and Levy 1984), in which lamellae are composed of slight shifts in microfibril orientation (10–20°, through a 180° arc), was first described in *Nitella* (Charophyceae; Probine and Barber 1966). In the charophycean order Zygnematales, the primary wall is structurally similar to that typical of vascular plants, but the secondary wall consists of distinct obliquely-intersecting bands of microfibrils (Mix 1972, 1973). An outer wall layer of mucilage may also be present in the Zygnematales. Furthermore, differences in cell wall composition between filamentous members of the Charophyceae *(Klebsormidium flaccidum* (Kütz.) Silva, Mattox et Blackwell), Chlorophyceae (*Ulothrix belkae* Mattox et Bold), Pleurastrophyceae (*Pleurastrum terrestre* Fritsch et John), and Ulvophyceae (*Pseudendoclonium basiliense* Vischer) led to the proposal that the origin of the green algal cell wall is polyphyletic (Domozych et al. 1980).

Charophycean algae (Mattox and Stewart 1984) are thought to be extant representatives of the first organisms to possess a cellulosic cell wall on the phylogenetic line that gave rise to vascular plants (Domozych et al. 1980). In this paper, we describe the cell wall composition of the charophycean alga *Mougeotia* and discuss its relationship to the cell wall composition of other algae and of vascular plants.

## MATERIALS AND METHODS

Unialgal *Mougeotia* sp. (UTEX #758; Starr and Zeikus 1987) cultures were maintained in *Volvox* medium (Provasoli and Pintner 1959, Starr and Zeikus 1987) with 10 mM HEPES buffer (pH 7) substituted for glycyl-glycine buffer and with the vitamin thiamine included. Cultures were maintained at 20° C, 16:8 h LD photoregime (4000 lux, General Electric cool white F40 fluorescent lights) and transferred to fresh medium every month. Large-scale growth of algal filaments was obtained in 500 mL culture flasks containing 200 mL of culture medium constantly aerated with bubbling air.

*Cell wall structure.* Cell walls in the native state and isolated free from cytoplasm were examined with polarization microscopy and in thin-section, freeze-fracture replica and shadowed preparations for electron microscopy. Filaments cleared of cytoplasm (80% ethanol) were observed with a Zeiss Universal microscope equipped with polarization optics, a 1/20 Brace-Kohler compensator and a red I retardation plate. Specimens fixed in 2% glutaraldehyde buffered with 0.2 M phosphate (pH 7), post-fixed with 0.1% $OsO_4$ (in 0.2 M phosphate buffer, pH 7), dehydrated in a graded acetone series and embedded in Spurr's resin, were thin-sectioned and post-stained with uranyl acetate and lead citrate prior to photography with a JEOL 100-C electron microscope (80 kV). Unfixed specimens were mounted in 2 mm double replica gold supports (Balzers) and frozen in liquid propane (cooled with liquid nitrogen). Frozen specimens were stored in liquid nitrogen prior to fracturing in a Balzers BA 360 M apparatus operating at −106° C and 1–2 × $10^{-6}$ Torr. Platinum/carbon (Pt/C) replicas were produced with an electron gun (2 kV, 90 mA, 6–7 s, 45°), following 30 s of etching. A carbon backing film was deposited on the replica by resistance coating. Replicas were cleaned in a 2.5% (w/v) Na dichromate/50% (w/v) $H_2SO_4$ solution. Pt/C shadowing of specimens spread on formvar-coated grids was performed as above except that a longer duration (10 s) electron gun burn was used. Freeze-fracture replicas and shadowed specimens were photographed with a Philips EM 420 electron microscope (80 kV). Micrographs were oriented with the direction of shadowing from below.

*Cell wall composition.* A whole wall preparation was isolated from *Mougeotia* by a modification of the procedure of Gretz et al. (1982). Cells were fragmented by Braun cell homogenization (5 × 1 min, 4000 rpm, 0.45–0.5 mm glass beads) in 10 mM HEPES buffer (pH 7, 4° C). Sequential fractionation of the whole wall preparation was performed as described previously (Gretz et al. 1982) except that the conditions for alkali extraction consisted of 1 N NaOH, 0.25 M $NaBH_4$, 100° C, 1 h, which yielded an insoluble residue. In addition, the chlorite extraction was omitted. An acid-modified fraction was obtained by limited hydrolysis (2.5 N HCl, 100° C, 15 min) of the hot alkali-extracted insoluble residue. The hot water-solubilized and hot alkali-solubilized fractions were fractionated further on DEAE Sephacel ($Cl^-$ form) columns (3.5 × 1.0 cm) according to Stevens and Selvendran (1984). One mL fractions were collected. Every fifth fraction was monitored for carbohydrate (phenol-sulfuric assay; Dubois et al. 1956) and acidic carbohydrate (carbazole assay; Bitter and Muir 1962, Selvendran et al. 1979). Fraction carbohydrate content was expressed as glucose, xylose or glucuronic acid equivalents. A mixture of xylan and polygalacturonic acid standards also was separated by this procedure for reference. Sulfate, pyruvic acid and protein assays of the *Mougeotia* cell wall were performed according to Craigie et al. (1984), Hirase and Watanabe (1972) and Lowry et al. (1951), respectively.

Monosaccharide analysis was performed by acid hydrolysis of cell wall fractions using two methods: A) treatment with fuming HCl for 10 min at 4° C, then 15 min at 25° C, followed by 2 N trifluoroacetic acid (3 h, 121° C) (Gretz et al. 1982); or B) treatment with fuming HCl 85 min at 4° C, then 25 min at 25° C, followed by 3.5 N HCl (14 h, 100° C). The former procedure provided optimal monosaccharide yields for non-cellulosic car-

bohydrates, whereas the latter procedure optimized yields for cellulosic carbohydrates. Alditol acetates were formed from cell wall fraction hydrolysates according to the methods of Blakeney et al. (1983) and separated on a Supelco SP-2330 fused silica capillary column (30 m, 0.25 mm ID, 240° C, isothermal) in a Varian 3700 gas chromatograph (GC) with flame ionization detection. Quantification was based on response factors derived from repeated injection of standards which had been subjected to the same hydrolytic procedure as the cell wall fractions (Sloneker 1972). Qualitative determination of the acidic monosaccharides present in cell wall fractions was performed according to Hicks et al. (1985). Acidic and enzymatic hydrolysates were separated on Biorad HPX-87H and HPX-22H (Hicks and Hotchkiss 1988) HPLC columns (0.4 $mL \cdot min^{-1}$, 0.01 N $H_2SO_4$ mobile phase) with UV detection at 215 nm.

Per-O-methylation of cell wall fraction polysaccharides was performed according to Harris et al. (1984) with premethylation of cellulosic samples. The procedures utilized for separation of per-O-methylated carbohydrates from the methylation reaction mixture, and their subsequent hydrolysis and reduction were reported by Waeghe et al. (1983). Acetylation of per-O-methylated alditols was according to Harris et al. (1984). Glycosyluronic acid-containing fractions were treated with Dowex 50W-x12 ($H^+$ form) prior to per-O-methylation (Waeghe et al. 1983). Following methylation and prior to hydrolysis, acidic carbohydrates were reduced by treatment with 1 M lithium triethyl borodeuteride in tetrahydrofuran for 1.5 h at 25° C (York et al. 1985). Per-O-methylated alditol acetates were separated on a SP-2330 column (30 m, 0.25 mm ID, 150–245° C at 4° $C \cdot min^{-1}$, held at 245° C for 20 min, injector 240° C). Mass spectra were obtained on a Finnigan-MAT 4023 gas chromatograph-mass spectrometer (GC-MS) which was operated at 3 kV in the electron ionization mode and used to detect column effluent. Glycosyl linkages were determined according to characteristic ions reported by Jansson et al. (1976) and Waeghe et al. (1983). The glycosyl linkage nomenclature used here is according to Darvill et al. (1980).

*X-ray diffraction.* Purified cellulosic fractions were analyzed by x-ray diffraction. Powder patterns (sample rotation = 1 rpm) were produced from 1–2 h exposures on a Philips PW 1729 x-ray generator (35 kV, 25 mA) with a Debye-Scherrer camera (114.83 mm diam) utilizing Ni filtered Cu $K_\alpha$ radiation. Dry samples were packed in glass capillaries (0.2 mm ID) prior to mounting in the camera.

## RESULTS

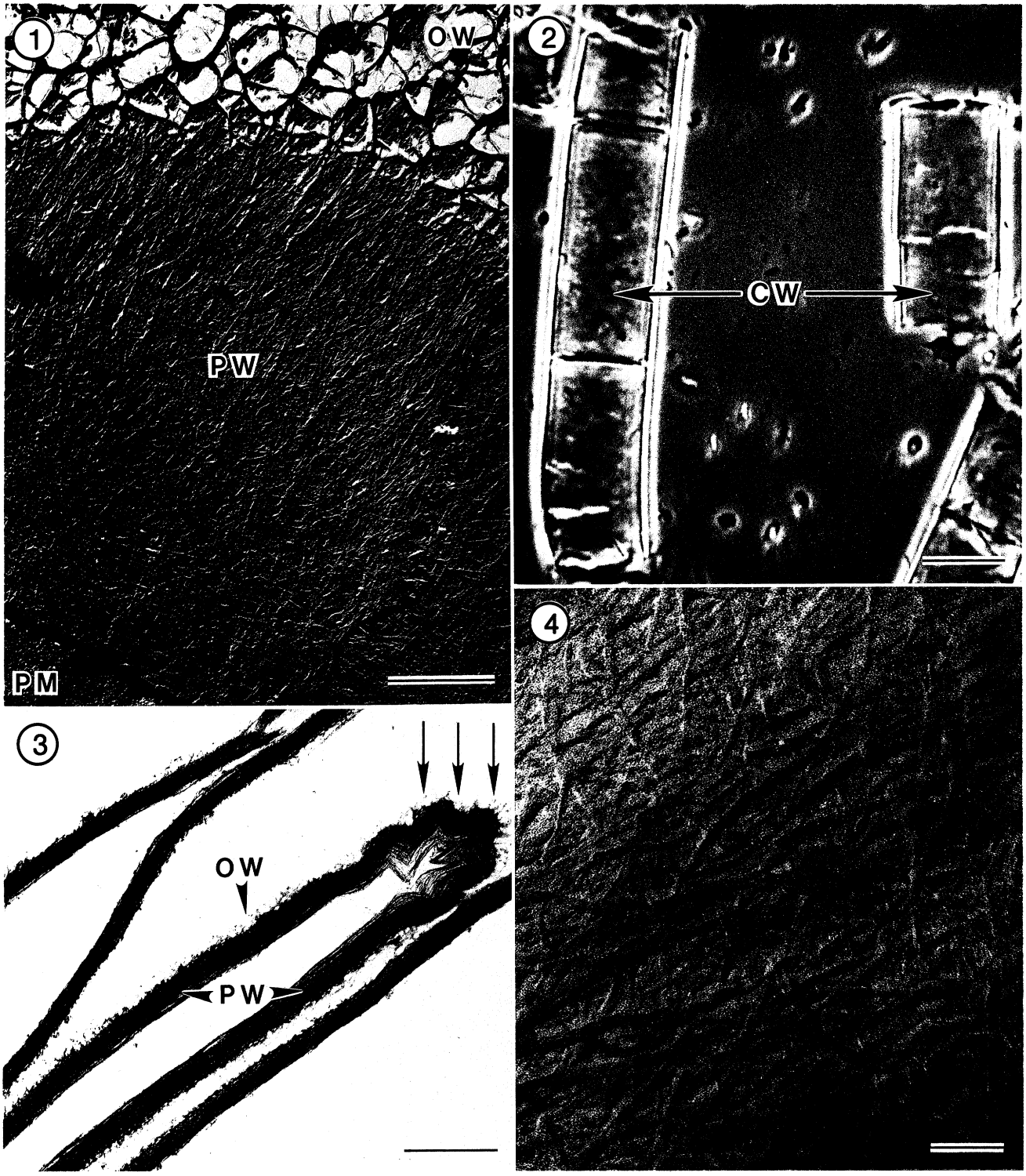
The *Mougeotia* cell wall consists of primary wall and mucilaginous outer wall layers (Fig. 1). The primary wall was composed of microfibrils (8 nm wide) preferentially oriented perpendicular to the cell long axis proximal to the plasma membrane, but oriented progressively more parallel to the cell long axis distal to the plasma membrane (Fig. 1). The outer wall layer was typically three times wider in cross-fracture than the primary wall and consisted of a loose mesh-work of randomly oriented anastomosing fibrils (Fig. 1). No evidence of the zygnematalean-type secondary wall (Mix 1972, 1973) was observed.

Polarization microscopy demonstrated that the overall microfibril orientation of *Mougeotia* cell walls was transverse to the cell long axis. A birefringence index of 0.85 and mean angle of dispersion of 27.92° was calculated from cylindrical cell walls according to Richmond (1983). A blue color was observed when the wall long axis was oriented perpendicular to the slow axis of the red I retardation plate and colored yellow when rotated 90°. Therefore, the sign of birefringence for *Mougeotia* cell walls was negative relative to the cell long axis. No differences in microfibril orientation were observed between different cells on the same filament.

The isolated whole wall preparation was determined to be free of cytoplasmic debris by light and electron microscopy (Figs. 2, 3). The homogenization and purification process was gentle enough to preserve the structural integrity of the cell wall in the whole wall preparation (Fig. 2). The fine structure of the preparation in thin section (Fig. 3) consisted of primary cell wall microfibrils with tufts of loosely arranged mucilage attached to the outer surface which appeared to adhere to the mucilage of adjacent wall fragments. The amount of mucilaginous matrix was sparsely represented in whole cell wall thin-sections compared to that observed in the freeze fracture of whole cells. Cross-walls were typically free from wall material of the adjacent cell (Fig. 3) as a result of filament fragmentation during homogenization. Fragmentation yielded a half cross-wall, consisting of folded, obliquely intersecting microfibrils at the ends of each generally rectangular cell wall fragment.

The whole cell wall preparation consisted of 77.8% carbohydrate (phenol-sulfuric assay), 7.3% acidic carbohydrates (carbazole assay), 2.9% "lipid" (weight loss during fractionation) and <1.0% protein (Lowry assay). The sulfate and pyruvic acid content of the whole cell wall preparation was 0.6% (Craigie et al. 1984 assay) and 0.7% (lactic acid dehydrogenase assay), respectively. The *Mougeotia* cell wall consisted of 63.6% noncellulosic carbohydrates (hot $H_2O$, hot 1 N NaOH and hot 2.5 N HCl soluble, phenol-sulfuric assay positive material) and 13.4% cellulose (the 2.5 N HCl insoluble residue was 14.5% of the whole wall weight and was almost exclusively 4-linked glucose). X-ray diffraction of the acid modified fraction demonstrated interlattice d-spacings of 0.602 nm, 0.535 nm and 0.396 nm, which are typical of cellulose I. This was the only *Mougeotia* wall fraction in which x-ray diffraction evidence for crystalline cellulose could be demonstrated, even though it represented 87% of the NaOH insoluble residue fraction by weight. The *Mougeotia* cellulose I allomorph was weakly crystalline (43%) compared to that found in *Boergesenia* (68%) and *Acetobacter* (65%) alkali insoluble fractions (Hotchkiss 1987). Mercerization (hot 5 N NaOH) of *Mougeotia* cellulose I produced d-spacings of 0.765 nm, 0.442 nm and 0.412 nm typical of cellulose II. The acid (2.5 N HCl) modified fraction also was observed to be fibrillar with Pt/C shadowing (Fig. 4).

Eight neutral sugars were present in the *Mougeotia* cell wall with glucose, xylose and galactose most abundant (Table 1). This included two unquantified 3-O-methyl-6-deoxyhexoses detected by mass spectrometry which were present in minor amounts. Variation in the total amount of monosaccharides




FIGS. 1–4. *Mougeotia* cell wall structure. FIG. 1. The freeze fractured native cell wall revealing a fibrillar, two-layered structure. Primary wall (PW) and outer wall (OW) layers surround the plasma membrane (PM). Scale bar = 0.5 μm. FIG. 2. The mechanically isolated cell wall (CW) preparation examined with phase-contrast microscopy during purification from cytoplasmic debris and glass particles. Scale bar = 25 μm. FIG. 3. Thin-sectioned cytoplasm-free wall fragments which were typical of material used for chemical analysis. The orientation of PW microfibrils was disrupted in end wall regions (3 arrows), which formerly were part of cross walls connecting adjacent cells. Scale bar = 1 μm. FIG. 4. The fibrillar structure of the acid-modified, alkali-insoluble wall fraction demonstrated by Pt/C shadowing. Scale bar = 0.1 μm.

TABLE 1. *Monosaccharide composition expressed as % fraction weight determined from alditol acetates derived from fraction hydrolysates, calculated as anhydro-aldose and given as $\bar{X}$ (range) with sample size ≥3 representing at least two independent hydrolysis runs (+ = values detected <1%).*

| Fraction | Glc | Xyl | Gal | Ara | Fuc | Rha[a] | Uronic acid[b] | Σ |
|---|---|---|---|---|---|---|---|---|
| Whole wall | 19.2[c] (17.5–22.1) | 11.7 (9.4–13.9) | 9.2 (8.2–10.3) | 2.0 (1.5–2.3) | 3.7 (3.3–4.3) | 2.1 (1.6–2.7) | 7.3 (6.2–7.9) | 55.2 |
| Hot $H_2O$ soluble | 7.1 (4.5–10.1) | 24.5 (16.0–32.6) | 13.7 (8.5–17.9) | 4.0 (2.5–5.0) | 5.9 (3.4–7.7) | 2.6 (1.7–3.1) | 10.6 (10.1–11.4) | 68.4 |
| Hot 1 N NaOH soluble | 1.0 (0.7–1.1) | 20.0 (15.3–25.8) | 14.1 (10.4–17.6) | 2.5 (2.4–2.8) | 2.8 (1.9–3.8) | 3.3 (2.1–4.1) | 9.7 (9.2–10.4) | 53.4 |
| Insoluble residue | 90.9[c] (81.1–97.8) | 1.5 (1.4–1.5) | + | + | + | | | 92.4 |
| 2.5 N HCl modified insoluble residue | 92.3[c] (91.6–93.4) | 1.8 (1.1–2.7) | | | | | | 94.1 |

[a] Peaks contained trace amounts of 3-O-methyl-6-deoxyhexose.
[b] Expressed as anhydro-glucuronic acid equivalents determined from carbazole assay corrected for neutral sugar content, representing glucuronic acid and galacturonic acid as determined by HPLC.
[c] Hydrolysis involved treatment with 3.5 N HCl (14 h, 100° C).

detected from run to run led to large range values in some cases (Table 1). When values were expressed as percent of total monosaccharide detected, the largest range observed was only 4% (xylose in the hot NaOH soluble fraction, 43.1–47.1%). Arabinose detected in *Mougeotia* cell wall fractions was consistently in the pyranose form (t-Ara = 1,5-di-O-acetyl-1-deuterio-2,3,4-tri-O-methylarabinitol) rather than the furanose form. Galacturonic and glucuronic acids were detected qualitatively by HPLC. Almost all of the acid modified fraction consisted of glucose (92.3%) with only trace amounts of xylose (1.8%) detected. The major monosaccharides present in the hot $H_2O$ and hot 1 N NaOH soluble fractions were xylose and galactose. Following DEAE Sephacel ($Cl^-$) anion-exchange chromatography, the hot $H_2O$ and hot 1 N NaOH soluble fractions were separated into a neutral subfraction and a subfraction which contained acidic constituents (Fig. 5). The occurrence of 4- and 2,4-linked xylosyl residues in the acidic subfraction of the xylan/polygalacturonic acid standard sample indicates that complete fractionation was not possible with these techniques. However, the neutral subfraction contained only xylosyl residues (no 4-galacturonosyl or 2,4-rhamnosyl linkages detected) and only negligible carbazole positive material.

Only major wall fraction glycosyl linkages are reported (Table 2) based on peak area and published response factors (Sweet et al. 1975). For example, the NaOH soluble neutral subfraction consisted mainly of 4-, and 2,4-xylosyl linkages, while several minor linkage peaks were also present (Fig. 6). Some of these minor linkages, such as t-arabinosyl, t-xylosyl and t-galactosyl, may be significant to the structure of the purified NaOH soluble neutral polymer. Significant minor linkages in the $H_2O$ soluble neutral subfraction included t-galactosyl and 6-galactosyl. The insoluble residue that remained after hot NaOH extraction and the 2.5 N HCl modified insoluble residue consisted of almost exclusively 4-glucosyl linkages. Although trace amounts of hemicellulosic linkages (t-, and 4-xylosyl; 4,6-glucosyl) remained relatively constant following acid

TABLE 2. *Glycosyl linkage composition expressed as the position(s) of substitution in addition to C-1 (i.e. t-hexose = 1,5-di-O-acetyl-1-deuterio-2,3,4,6-tetra-O-methylhexitol), determined from per-O-methylated alditol acetates derived from cell wall fractions or DEAE Sephacel ($Cl^-$) fractionated cell wall fractions.*

| Fraction | Subfraction | Glc | Xyl | Gal | Ara | Fuc | Rha | GlcA | GalA |
|---|---|---|---|---|---|---|---|---|---|
| Hot $H_2O$ soluble | Acidic | | 4-<br>2,3-<br>2,4-<br>3,4- | 2- | 4- | t- | t- | 4- | |
| | Neutral | 4- | | 3- | 2,4- | t- | | | |
| Hot NaOH soluble | Acidic | | 4-<br>2,3-<br>2,4-<br>3,4- | t-<br>2-<br>6- | | t- | t-<br>2,4- | | 4- |
| | Neutral | | 4-<br>2,4- | | | | | | |
| Insoluble residue | | 4- | | | | | | | |
| 2.5 N HCl modified insoluble residue | | 4- | | | | | | | |

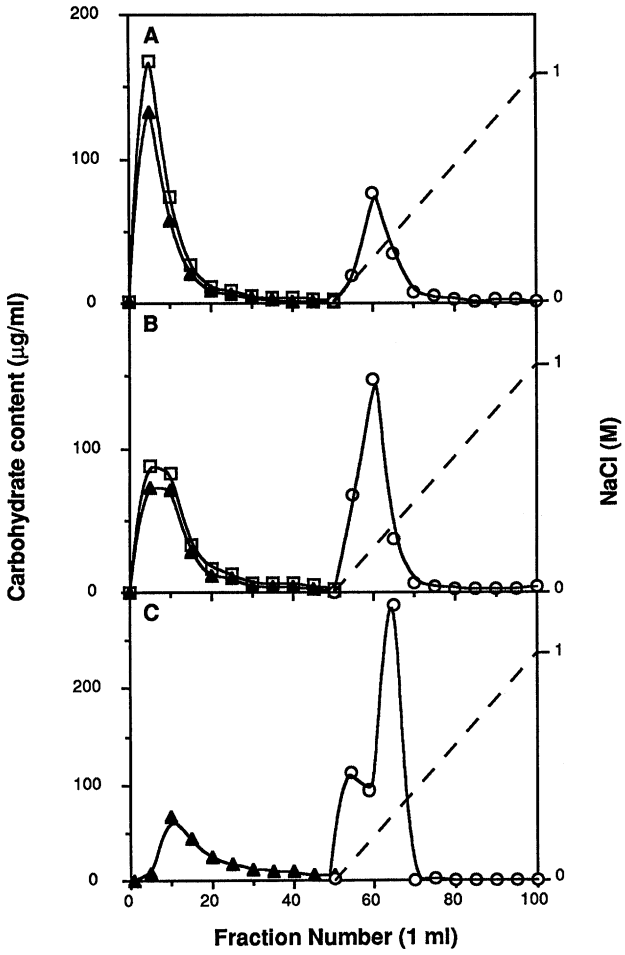



FIG. 5. Fractionation by DEAE Sephacel anion-exchange chromatography of (A) *Mougeotia* hot water soluble wall fraction, (B) *Mougeotia* hot alkali soluble wall fraction, and (C) a mixture of xylan and polygalacturonic acid standards. Elution of 10 mg sample loads was with 10 mM phosphate buffer (pH 6.4) for the first 50 mL followed by a linear gradient of NaCl (– –) in phosphate buffer. Carbohydrate content was expressed as glucose (□), xylose (▲) or glucuronic acid (○) equivalents.

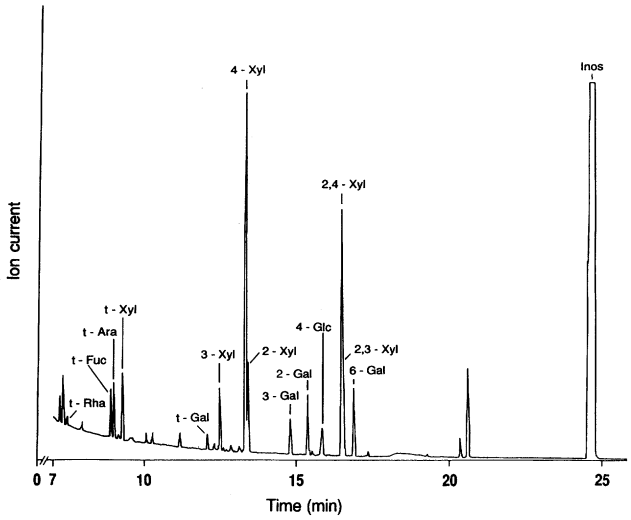



FIG. 6. Total ion chromatogram observed from GC-MS of per-O-methylated alditol acetates derived from the *Mougeotia* hot alkali soluble neutral subfraction. Glycosyl linkages, determined from mass spectral fragmentation patterns, are indicated (t-Rha = terminal rhamnosyl, 4-Xyl = 4-xylosyl) for corresponding peaks. Inositol hex-acetate (Inos) was included as an internal standard.

modification, the amount of 4-glucosyl linkages in the acid modified fraction was significantly greater than in the unmodified alkali insoluble residue.

## DISCUSSION

The *Mougeotia* primary wall structure is similar to that typical of most plants and fungi with cellulosic cell walls. Although the innermost cell wall microfibrils appeared to be almost randomly oriented in freeze fracture preparations, an overall transverse microfibril orientation was demonstrated by polarization microscopy. The microfibril orientation in *Mougeotia* cell walls was more transverse and less disperse than that reported for *Nitella* (0.85 vs. 0.7 birefringence index, 28° vs. 36° mean angle of dispersion; Richmond 1983). In *Nitella*, the microfibril orientation changes during cellular development from random in the apical cell wall to proximally transverse and distally axial in elongating cell walls (Richmond 1983) and finally helicoidal in mature cell walls (Hotchkiss and Brown 1987). The absence of intercellular developmental changes in microfibril orientation in *Mougeotia* may be due to a generalized pattern of growth in which cell divisions can occur at all points on the filaments (Hotchkiss 1987).

The pattern of microfibril orientation in *Mougeotia* primary walls is consistent with the multinet growth hypothesis (Roelofsen and Houwink 1953) which proposes that inner transversely oriented microfibrils become passively reoriented to an axial arrangement distal to the plasma membrane due to the strain of elongation growth (Preston 1982). Our results suggest that the pattern of microfibril orientation of the primary wall is not lamellate, crossed-helical (Roland et al. 1975) or helicoidal (Neville and Levy 1984). With shadowed (Buer 1964) and thin-sectioned (Foos 1971) preparations, *Mougeotia* cell walls were found to consist of two layers, an inner layer (primary wall) of transversely oriented microfibrils and an outer colloidal layer (also fibrillar), which also was negatively birefringent (Buer 1964). Our results confirm the proposal made by Foos (1971) concerning changes in the microfibril orientation from the inner to outer primary wall. Foos (1971) also reported a middle lamella (loosely arranged fibrils) which connected adjacent primary walls in cross-wall structures. The absence of a secondary wall in *Mougeotia* contradicts the proposal of Mix (1972, 1973) who suggested that all members of the Zygnemataceae contain secondary walls in addition to primary and outer mucilage wall layers. Our evidence suggests that cell wall taxonomic char-

TABLE 3. *Cell wall composition as % dry wall weight.*

| | *Mougeotia* | *Klebsormidium flaccidum*[a] |
|---|---|---|
| Hot $H_2O$ soluble carbohydrate | 35.0[b] | 38.0 |
| Hot NaOH soluble carbohydrate | 26.8[b] | 29.0 |
| Cellulose | 13.4 | 6.7 |
| Total pentose | 8.1 | 10.0 |
| Total rhamnose | 2.1 | 3.0 |
| % Rhamnose in hot $H_2O$ soluble fraction | 2.6 | 7.9 |
| % Glycuronic acid in wall | 7.3 | 11.6 |
| % Glycuronic acid in hot $H_2O$ soluble fraction | 10.6 | 30.5 |

[a] From Domozych et al. (1980).
[b] Expressed as glucose equivalents determined by phenol-sulfuric acid assay.

acteristics of the Zygnemataceae should be revised to include members lacking secondary wall formation.

Glucose was the major cell wall monosaccharide in the *Mougeotia* cell wall preparation. Most of the glucose was derived from cellulose which was identified by chemical and x-ray diffraction analysis. The presence of cellulose in *Mougeotia* was first reported by Tiffany (1924) based on solubility in cuprammonium. Disagreement has existed concerning x-ray diffraction evidence for the presence of cellulose I in zygnematalean cell walls (Nicolai and Preston 1952, Kreger 1957, Preston 1974). However, we observed that the *Mougeotia* cellulose interlattice d-spacings were not significantly different from those typical of cellulose I according to the Senti and Zobel (1962) convention when the duration of acid modification was extended (24 h, 1% HCl, 25° C; Hotchkiss 1987). Mercerization of *Mougeotia* cellulose provides additional evidence for the presence of cellulose I in the native state.

Acid-modification of the *Mougeotia* insoluble residue fraction removed trace amounts of xylose, galactose, arabinose and fucose. The same treatment (2.5 N HCl) also may have selectively removed noncrystalline 4-linked glucan from the cellulose, thereby lowering the degree of polymerization and allowing for more efficient derivatization during linkage analysis. This could explain why significantly more 4-linked glucan was detected in the acid-modified fraction relative to the alkali insoluble residue fraction. Past disagreement concerning the presence of cellulose I in the Zygnematales probably was due to the inability to completely remove hemicellulosic carbohydrates or noncrystalline glucan, which limited the association of cellulose crystallites to a size smaller than that necessary for detection by x-ray diffraction.

The major hemicellulosic (hot 1 N NaOH soluble) neutral polysaccharide of *Mougeotia* cell walls was a 4-linked xylan with 2,4-xylosyl branchpoints. Minor linkages also present in this subfraction suggest that the xylan may be an arabinoxylan similar in structure to the major hemicellulose of monocotyledonous plants (McNeil et al. 1975, Darvill et al. 1980, McNeil et al. 1984). Xylan is not an uncommon cell wall constituent in algae. However, in those green algae investigated (members of the Ulvophyceae), the xylans were 3-linked and unbranched (Preston 1974, Percival and McDowell 1981). Water-soluble mixed 3-, 4-linked xylans (Barry and Dillon 1940, Turvey and Williams 1970) and alkali soluble 4-linked xylans (Gretz et al. 1987) have been reported in the red algae.

The major hot water soluble neutral polysaccharide in *Mougeotia* was a 3-linked galactan. The presence of 4- and 2,4-arabinosyl linkages in this subfraction suggests that an arabinogalactan structurally similar to one of the pectic carbohydrates found in primary walls of dicotyledonous plants (Darvill et al. 1980) may be present. In those green algae in which 3-linked galactans have been described (Ulvophyceae), the polysaccharides contain sulfate esters (Percival and McDowell 1981), while the *Mougeotia* galactan lacks significant amounts of esterified sulfate. The presence of a hot $H_2O$ soluble neutral 4-linked glucan may indicate starch contamination, although starch grains were not observed in thin sections of isolated wall fragments. The partially purified acidic polysaccharides of the *Mougeotia* cell wall yielded a complex mixture of linkages. Glucuronoxylorhamnans have been reported as the major acidic matrix polysaccharides of several marine green algal cell walls (Percival and McDowell 1981); however, these glucuronoxylorhamnans are also highly sulfated.

*Klebsormidium* (Domozych et al. 1980) is the most closely related green alga to *Mougeotia* with which comparisons based on cell wall composition can be drawn. Relative to members of the Chlorophyceae, Ulvophyceae and Pleurastrophyceae examined by Domozych et al. (1980), the cell walls of *Mougeotia* and *Klebsormidium* are composed of abundant water soluble and alkali soluble polysaccharides and have high total pentose and low water soluble rhamnose (Table 3). In addition, *Mougeotia* has the lowest percent uronic acid in the water soluble fraction, while *Klebsormidium* possesses the lowest cellulose content (6.7%) compared to the other algae analyzed previously (Domozych et al. 1980). Therefore, it appears that the cell walls of both *Mougeotia* and *Klebsormidium* are very similar in composition even though the techniques used for analysis differed. Domozych et al. (1980) used the characteristics of low cellulose content, low water soluble rhamnose, and abundant water and alkali soluble carbohydrate to distinguish *Klebsormidium* from members of the other green algal classes. They also proposed, based on comparative cell wall structure and composition, that *Klebsormidium* represents a position on the charophycean phylogenetic line soon after the evolution of the cellulosic cell wall.

The cell wall composition of advanced members

of the Charophyceae such as *Nitella* and *Chara* consists of higher cellulose content (20–26%) and relatively less significant pectic and hemicellulosic polysaccharide content (Anderson and King 1961a, b, Métraux 1982) than do *Mougeotia* and *Klebsormidium* cell walls. The degree of methyl esterification of *Nitella* and *Chara* pectins was negligible (0.5%; Anderson and King 1961a, b) which necessitated the use of chelating agents such as ammonium oxalate and ethylenedinitrilotetraacetic acid for efficient pectin extraction (Anderson and King 1961a, b, Métraux 1982). An evolutionary continuum of cell wall compositional complexity may exist in the Charophyceae with *Mougeotia* and *Klebsormidium* representing the primitive condition whereas *Nitella* and *Chara* would be typical of the advanced condition.

Although the composition of the *Mougeotia* cell wall appears to be similar to that of vascular plants, at least two characteristics distinguish the former from the latter. Arabinose in vascular plant cell walls typically is in the furanose form (Darvill et al. 1980) rather than the pyranose form as in *Mougeotia.* The occurrence of two 3-O-methyl-6-deoxyhexoses in the *Mougeotia* cell wall is also unusual. Methylated sugars have been reported as rare constituents of vascular plant cell wall polysaccharides (Darvill et al. 1980). Rhamnogalacturonan II isolated from primary walls of dicotyledonous plants contains 2-O-methyl-xylose and 2-O-methyl-fucose in minor amounts (Darvill et al. 1980). Furthermore, 4-O-methyl-xylose was reported as a constituent of *Chlorella* (Chlorophyceae) cell walls (Brunner and Loos 1985) and 4-O-methyl-galactose was observed in *Acetabularia* (Ulvophyceae) cell walls (Percival and McDowell 1981).

Based on cell wall structure and composition, the *Mougeotia* cell wall, like that of *Klebsormidium,* represents a primitive state in the evolution of the cellulosic wall. Certain elements of this cell wall composition indicate greater homologies with vascular plant cell walls than with green algal (chlorophycean, pleurastrophycean or ulvophycean) cell walls described to date. The evidence supports proposals based on ultrastructural (cytokinetic apparatus, flagellar apparatus, motile cell scales, terminal cellulose synthesizing complexes) (Mattox and Stewart 1984, Hotchkiss and Brown 1987) and biochemical (glycolate oxidizing and urea degrading enzymes) (Frederick et al. 1973, Syrett and Al-Houty 1984) characteristics that the Charophyceae represents the phylogenetic line that gave rise to vascular plants. The *Mougeotia* cell wall is not identical to vascular plant cell walls, but the former probably represents the primitive condition from which the latter evolved.

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